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(71) Applicants International remote imaging systems Inc. (USA California), 9232 Deering Avenue, Chatsworth, California 91311, United States of America.
(72) Inventors Sherman E DeForest, Gunner Bolz.
(74) Agent and/or Address for Service Reddie & Grose, 16 Theobalds Road, London WC1X 8PL.

(54) **A method of separating particles in a moving fluid sample**

(57) A method of separating particles in a fluid sample is disclosed. The fluid sample moves through a flow cell 10 which has a width and a thickness, each perpendicular to the direction of flow. The fluid sample 26 is conveyed through the flow cell 10 while, at the same time, a sheath fluid 22 is conveyed through the flow cell 10 in the same direction. The particles 70, 72 are aligned substantially with the minimum cross-sectional area extended transverse to the direction of flow and their maximum cross-sectional area extended substantially parallel to the width. A force C is imparted onto the fluid sample to separate the particles 70, 72, in accordance with a physical characteristic of the particles 70, 72 (eg size + hydrodynamic force, weight and gravity, electrical force, etc.)

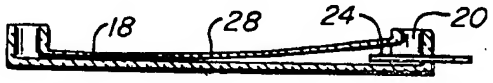

FIG. 3.

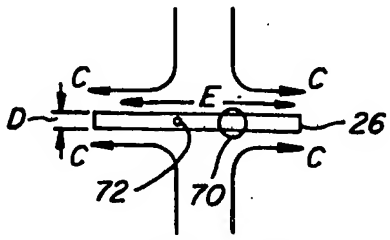

FIG. 5c.

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The drawings originally filed were informal and the print here reproduced is taken from a later filed formal copy.

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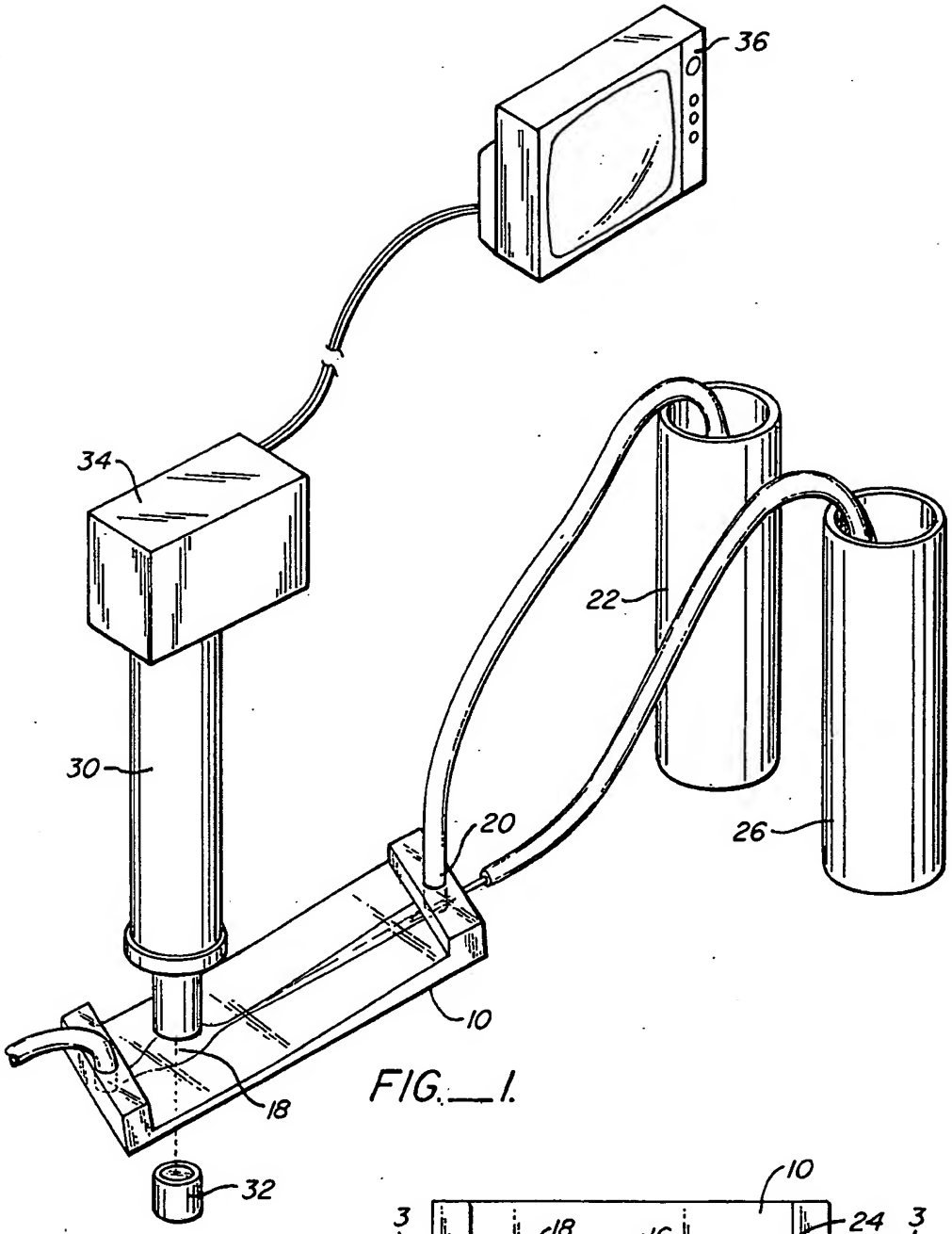

FIG. 1.

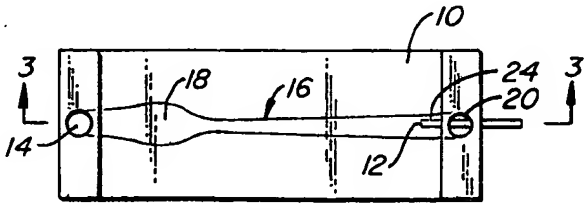

FIG. 2.

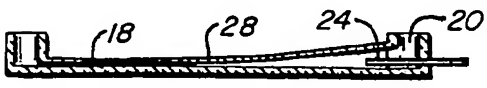

FIG. 3.

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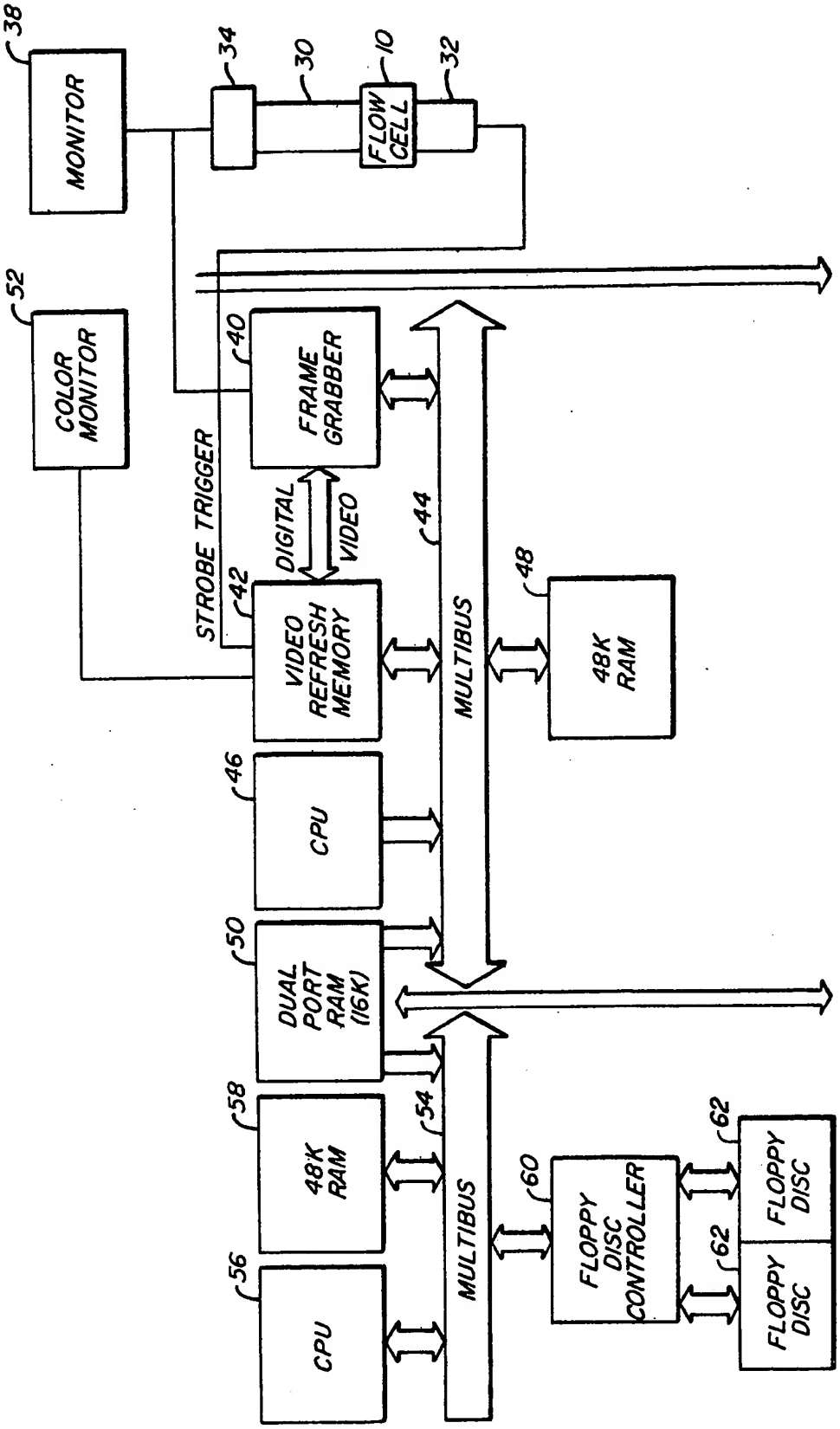

FIG. 4.

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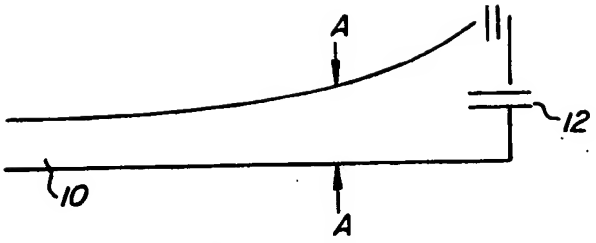

FIG._5a.

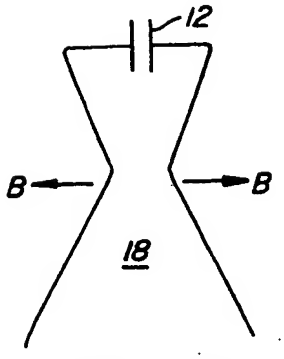

FIG._5b.

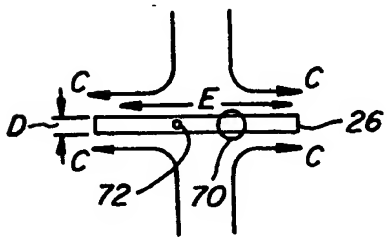

FIG._5c.

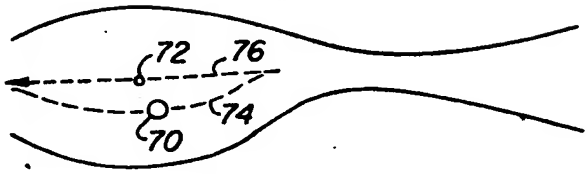

FIG._5d.

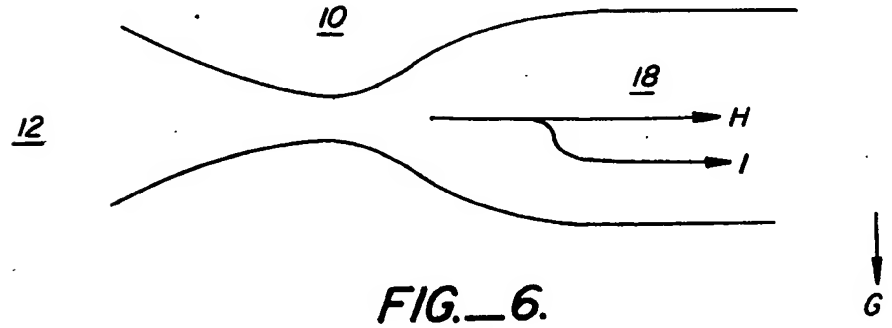

FIG._6.

SPECIFICATION

A method of separating particles in a moving fluid sample

Substantial advances have been made in automating the job of counting blood cells in a serum sample. The most well-known instrument for performing blood counts is the so-called Coulter counter in which blood cells are passed in single file through an orifice and detected and counted by the manner in which they change the electric properties at the orifice. Up until the present time, however, there has been no automated equipment available for analyzing and evaluating the multiple cells, such as normal cells, target cells, sickle cells, etc which may be found in a flowing stream of a given blood sample. Thus, where multiple cell information of this type is desired, the standard commercial way of obtaining it is by preparing a microscope slide with the cells fixed on an image plane and having a human operator or pattern recognition machine count statistically significant numbers of the cells as the cells are observed one-at-a-time on the slide through a microscope. See US Patent No 4,175,860 and 4,199,748.

Some attempts have been made in recent years to provide optical analysis of particles flowing in a flow stream. For instance, Kay, et al, *Journal of Histochemistry and Cytochemistry,* Volume 27, page 329 (1979) shows a Coulter type orifice for moving cells in single file with the cells magnified on a vidicon. Additionally, Kachel, et al., *Journal of Histochemistry and Cytochemistry,* Volume 27, page 335, shows a device for moving cells in single file through a microscopic area where they are photographed. While these workers have done some work in automating particle analysis in single file, no successful work has been reported where automated particle analysis was accomplished in a flowing stream without the requirement of arranging the particles into a single file stream. See, for instance, *Flow Cytometry and Sorting,* Melaned, et al., John Wiley & Sons 1979, Chapter 1; see also U.S. Patent No. 3,819,270.

Summary of the invention

In the present invention, a method is provided for separating particles flowing in a fluid sample. The fluid sample is moving in a direction through a flow cell, with the cell having a width and a thickness, each perpendicular to the direction of flow. The method of the present invention comprises the steps of conveying the fluid sample through the flow cell such that the particles are aligned substantially with their minimum cross-sectional area extended transverse to the direction of flow and the maximum cross-sectional area extended substantially parallel to the width. A sheath fluid also flows through the flow cell in the same direction. Finally, a force is imparted on the fluid sample to separate the particles in accordance with a physical characteristic of the particles.

Brief description of the drawings

Figure 1 is a perspective view of an apparatus for examining a flow stream in accordance with this invention.

Figure 2 is a plan view of the flow chamber in Figure 1.

Figure 3 is a cross-sectional view of the apparatus of Figure 2 taken on the plane indicated at 3-3.

Figure 4 is a schematic diagram of the electronic processor employed by the apparatus of Figure 1.

Figure 5A is a partial schematic side view of the flow chamber showing the force of constriction "A" in the thickness direction; *Figure 5B* is a partial, schematic top view of the flow chamber showing the force "B" in the width direction; *Figure 5C* is a partial, schematic, cross-sectional view of the flow chamber showing the resultant forces acting upon the fluid sample; *Figure 5D* is a partial, schematic top view of the flow chamber showing the trajectory of a large particle separated from the main stream of particles in accordance with the method of the present invention.

Figure 6 is a partial, schematic side view of the trajectories of particles separated in accordance with the method of the present invention.

Detailed description of the drawings

Referring now in detail to the drawings, and particularly to Figure 1, the apparatus shown therein includes a body 10 containing a flow chamber having an inlet 12 for a fluid sample, such as blood or urine, and an outlet 14 with a passageway 16 extending between them past an imaging area 18. The passageway 16 has an inlet with a conduit 20 adapted to be connected to a volume of saline solution 22. As illustrated in Figures 2 and 3, the inlet 12 for the fluid sample has a needle 24 in the passageway 16 downstream from the conduit 20 with the needle 24 connected to a container 26 adapted to hold the fluid sample to be analyzed.

From the inlet 12 to the imaging area 18, the cross-sectional area becomes progressively smaller. The thickness also decreases from the inlet 12 to the imaging area 18. The width decreases from the inlet 12 and then increases substantially to the imaging area 18. Thus, as illustrated in Figures 2 and 3, the passageway 16 has a width and depth of about 5,000 microns at the inlet 12 and a width and depth of about 500 microns at midpoint 28, and a depth of 100 microns with a width exceeding 5,000 microns at the examination area 18.

It will be appreciated that the flow stream through the examination area 18 can be many times deeper than the largest cells with the width many times wider than the widest particles, such as more than one hundred times as wide as the widest particle. However, with the flow passageway shaped in this way, the stream entering through the opening 12 is confined to a stable flow path of minimum shear in the examination area 18. Preferably, the cross-sectional area of minimum shear is not substantially larger than the minimum cross-sectional area of the particles whereby the particles are aligned in the flow stream with their minimum cross-sectional area extended transverse to their direction of flow and their maximum cross-sectional area parallel to the

width (i.e. visible in the plane of Figure 2). The term "minimum shear" is used herein to mean "minimum velocity gradient" so that a particle moving in the stream tends to align itself with the direction of the stream much as a log floating down a river will align itself with the direction of flow where there is a flow gradient. The flow characteristics in the passageway 16 may be controlled by adjusting the fluid pressure in container 22 and 26 either automatically or by adjusting the static heights thereof.

A microscope 30 is focused on the examination area 18 and the examination area 18 is illuminated from below by a strobe light 32 which is preferably a U.S. Scientific Instrument Corporation Model 3018 containing a 2UP1.5 lamp. The output of the microscope 30 is focused on a CCD camera 34 which is perferably a CCD camera model number TC1160BD manufactured by RCA Corporation. The output of the CCD camera 34 is converted to a series of still frame images, and suitable electronic processors are employed for evaluating those images. One processor which may be employed is the processor marketed as Image Analysis System Model C-1285 by Hamamatsu Systems, Inc., Waltham, Massachusetts. Preferably, the output of the CCD camera 34 is connected to an electronic processor 36 which is illustrated in greater detail in Figure 4 and includes a black and white television monitor 38 and a frame grabber 40 which stores still frame images of the subject viewed by the CCD camera. The frame grabber 40 is preferably a Model FG08 frame grabber made by the Matrox Corporation of Montreal, the output of which is supplied to a video refresh memory 42 Model RGB 256 made by Matrox Corporation which are both coupled to the multibus 44 of the central processing unit 46 which is preferably an Intel 80/20 computer. The multibus 44 is also coupled to a 48K random access memory 48 of Electronic Solutions, Inc., and a 16K dual port random access memory 50 Model RM 117 of Data Cube Corporation. The output of the video refresh memory is also coupled to a color monitor 52 which may be used to provide digitally enhanced video images of individual still frames for human examination.

The second output of the dual port ram 50 is connected to a multibus 54 which is connected to an Applied Micro Devices central processing unit 56, a 48K random access memory of Electronic Solutions, Inc. 58 and removable storage in the form of a floppy disc controller 60, such as an Advanced Micro Devices Model 8/8 and two units of Shugart floppy disc storage 62.

A wide variety of programming may be employed for processing pictures with the apparatus of Figure 4 depending upon the particular task which user wishes to perform.

As mentioned above, the programming of the Hamamatsu System 1285 may be employed. Preferably, however, the programming is performed as follows:

The tasks are first divided into those which must address each pixel in a given image and those which only address a small subset of the total. Since much time will be spent in the first class of tasks, they are programmed in assembly language on the interface processor 46 (the Intel 80/20 in Figure 4). The output of these operations are then transferred to the host machine 56 via the dual ported ram 50. On the host side, almost all of the necessary programming is more suitably done in a high level language such as Pascal (BASIC or FORTRAN could in principal be used also). The types of tasks that are done in the assembly language includes greyscale transformations, convolutions, and greyscale histogram calculations. The types of task done on the host side include overall control of the other devices, identification and segmentation of object of interest in the field of view, calculation of parameters associated with objects thus found, and formating the output of results. Another way of considering this separation of tasks in this fashion is that tasks which must be performed at speeds faster than a human operator are done in assembly. Tasks which are either complicated or which can operate at less than the maximum speed can be programmed in the higher language. Objects are found in a field of view primarily by setting a greyscale window function for values known to be characteristic of the desired object. These values can be established by prior knowledge or by well-known histogram techniques. When a pixel belonging to an object has been located in the field of view, an edge tracing program is invoked to outline the whole object associated with that pixel. Once the edge has been found, then many relevant parameters such as location, area, integrated optical density, and various moments can easily be calculated. Probability of membership in previously defined subgroups can be determined from these derived parameters by means of standard decision theory. Definitions of cell morphoplogy classifications are established by trained observers. These definitions are then used as the basis of the selected algorithms. Accuracy of the method is determined by comparison of machine results with those of trained observers examining the same samples. Output of the results can be programmed to be any of a variety of formats. Histograms, line plots, and tabular summaries are available for particular needs.

It is thus seen that with the flow cell 10, the stream of particles in the imaging area 18 is of two dimensions. Therefore, more than one particle can be examined in a single field, and different particles can be optically distinguished with a number of important advantages. For instance, two cells flowing together can be optically recognized whereas a Coulter counter could recognize them as a single double-sized cell. In addition, the still frame images can be enhanced with the digital image enhancement techniques which have been developed for satellite pictures and the individual frames may be analyzed to provide data on individual cells. For a blood sample, information such as size, cross-sectional area, shape, (circular cell, target cell, sickle cells, etc.), optical density, hemoglobin content on the cell basis, etc. can be obtained. Not only can individual cells be analyzed and optically sorted in this way, but, additionally, when the cells are so analyzed and sorted, different types of cells may be

individually counted to give automatically and at a single pass, the number of normal red cells per volume of sample, the number of target red cells per cc of sample, the number of sickled red cells per cc of sample, the number of white cells, the number of platelets, etc. per cc of sample.

Once a series of still frame images is prepared in digital form, a wide variety of very sophisticated information can be obtained about the particles in the series of images depending upon the complexity of computer equipment and software which may be used for analysis of the images.

Preferably, information derived from still frame images is combined to provide composite information reflecting the content of the multiple still frame images and/or predetermined reference images, and the composite information thus obtained may be used in a variety of ways. Thus, in simple systems, the information may be printed out, for instance, to advise a hematologist about composite measurements made from a blood sample. In more complex systems, the composite measurements may be used by process control, such as pressure in a homogenizer, temperature in a crystallizer, or nutrient feed rate in a microbial culture where the system monitors particle size or number.

Thus, it will be noted that the apparatus may be used for analysis of a variety of optically perceptible particles moving in a stream, both biological particles, such as cells in blood or cells, bacteria, casts and crystals in urine or particles in gas analyzers, etc., and the output of these measurements may be employed for process control, such as dispensing nutrients into a stream contaning microorganisms as mentioned above, the control of the growth of polymers and crystals, etc.

Figure 5A is a partial schematic side view of the flow chamber 10. The flow chamber 10 has a thickness which decreases from the inlet 12 to the imaging area 18, as previously discussed. This creates a constriction upon the fluids, both the sheath fluid as well as the sample fluid, as they flow from the inlet 12 to the imaging area 18. This force of constriction is shown by the arrows "A". Referring to Figure 5B, there is shown a partial, schematic top view of the flow chamber 10. Figure 5B shows the width decreasing from the inlet 12 and then increasing substantially to the imaging area 18. This creates a force of expansion which impacts the sheath fluid and the fluid sample and is shown by the arrows "B". In Figure 5C, which is a cross-sectional view of the flow chamber 10 looking from the imaging area 18 to the inlet area 12. The forces of constriction in the thickness and of expansion in the width are shown by the arrows "C". The forces "C" are the resultant forces of "A" and "B". The forces "C" confine and shape the fluid sample 26 to a stream having a cross-sectional area which is substantially rectangular in shape, with a thickness of "D" and a width of "E". It should be emphasized that the thickness "D" is extremely small and is on the order of 0.1 to tens of microns.

In the method of the present invention, the size of the sample fluid 26, i.e. its cross-sectional area, can be varied by regulating the rate of flow of the fluid sample 26, or the rate of flow of the sheath fluid, or of the ratio of flow of the fluid sample to the sheath fluid. In particular, the flow rates of either the sheath fluid, the fluid sample, or both can be adjusted such that the thickness "D" of the fluid sample 26 can be made smaller than the larger particles such as those shown in particle 70. In the event the size of the particle 70 is larger than the thickness "D" of the fluid sample 26, the hydrodynamic forces "C" caused by the particular shape of the flow cell and of the flow of the sheath fluid will impact the particle 70, pushing it away from the main stream of particles towards the periphery of the flow chamber 10. On the other hand, for smaller size particles, such as particles 72, i.e. particles having sizes smaller than the thickness "D" of the fluid sample 26, the hydrodynamic forces "C" will not affect these particles 72. The trajectory of the smaller size particles 70 which remain unaffected by the hydrodynamic forces "C" is shown as 76, while the trajectory of the larger size particles is shown as 74. The result is shown in Figure 5D. With the larger size particles sent along a different trajectory, the microscope 30 can then be used to focus on either the larger size particles 70 or on the trajectory containing the smaller size particles. It should be emphasized that the definition of the "size" of a particle in the context of the size being affected by the hydrodynamic forces does not necessarily mean the largest dimension. In fact, due to the geometry of the flow cell 10 as previously described, particles flowing in the flow cell 10 will be oriented with their minimum cross-sectional area transverse to the direction of flow and the maximum cross-sectional area parallel to the width. Therefore, the larger "size" particles 70, which are affected by the hydrodynamic forces "C" are those particles having a dimension in the minimum cross-sectional area plane, which is transverse to the maximum cross-sectional area plane and is larger than the thickness "D" of the fluid sample 26. The smaller size particles 72 are those particles having a dimension in the minimum cross-sectional area plane, which is transverse to the maximum cross-sectional area plane and is smaller than the thickness "D".

Referring to Figure 6, the flow cell 10 is shown being used to separate particles based upon their density. In this embodiment, the flow cell 10 has a width many times its thickness and is operated such that the width is parallel to the force of gravity as shown by the arrow "G". When the stream of particles is flowing from the inlet 12 to the imaging area, the main stream would travel along the trajectory shown by the arrow "H". The denser particles, however, are separated from the main stream and are acted upon by the gravitational force G and sink to a lower trajectory and move in that trajectory, shown by the arrow "I". Due to the force of gravity upon the particles, the denser particles are influenced more than the less dense particles and, as a result, the denser particles travel in a trajectory which is lower than the trajectory travelled by the main stream of particles. In this manner, separation of particles based upon density can be achieved.

Finally, it whould be emphasized that other physic-

al characteristic of the particles can be the basis for separation based upon a force imparted to the stream of sample fluid. For example, certain particles can be electrically charged before they are entered into the flow chamber 10. An electric field can be established parallel to the width or of the thickness. With the application of the electric field, the charged particles will be deflected from the main stream of particles. In this manner, separation of particles based upon electrical charge can be effected. Although this is analogous to electrophoresis, the movement of the particles in the stream is much more rapid. Moreover, the use of a sheath fluid in the method of the present invention is necessary to stabilize the flow of the fluid sample and to maintain the flow within the laminar range, i.e., without any turbulence. Similarly, separation of particles based upon magnetic permeability can be effected by a magnetic force.

CLAIMS

1. A method of separating particles in a fluid sample, moving in a direction through a flow cell, said cell having a width and a thickness, each perpendicular to said direction, said method comprising the steps of:

conveying said fluid sample through said flow cell, such that said particles are aligned substantially with their minimum cross-sectional area extended transverse to said direction and their maximum cross-sectional area extended substantially parallel to said width;

flowing a sheath fluid through said flow cell in said direction;

imparting a force on said fluid sample to separate said particles in accordance with a physical characteristics of said particles.

2. The method of Claim 1, wherein said force is hydrodynamic force and said physical characteristic is size.

3. The method of Claim 2, wherein said imparting step comprises:

adjusting the rate of flow of said fluid sample.

4. The method of Claim 2, wherein said imparting step comprises:

adjusting the rate of flow of said sheath fluid.

5. The method of Claim 2, wherein said imparting step comprises:

adjusting the ratio of the rate of flow of said fluid sample to the rate of flow of said sheath fluid.

6. The method of Claim 1, wherein said width is many times said thickness.

7. The method of Claim 6, wherein said force is gravitational force and said physical characteristic is density.

8. The method of Claim 7, wherein said imparting step comprises:

orienting said flow cell with said width substantially parallel to said gravitational force.

9. The method of Claim 1, wherein said force is electrical and said physical characteristic is electrical charge.

10. The method of Claim 1, wherein said force is magnetic and said physical characteristic is magnetic permeability.

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